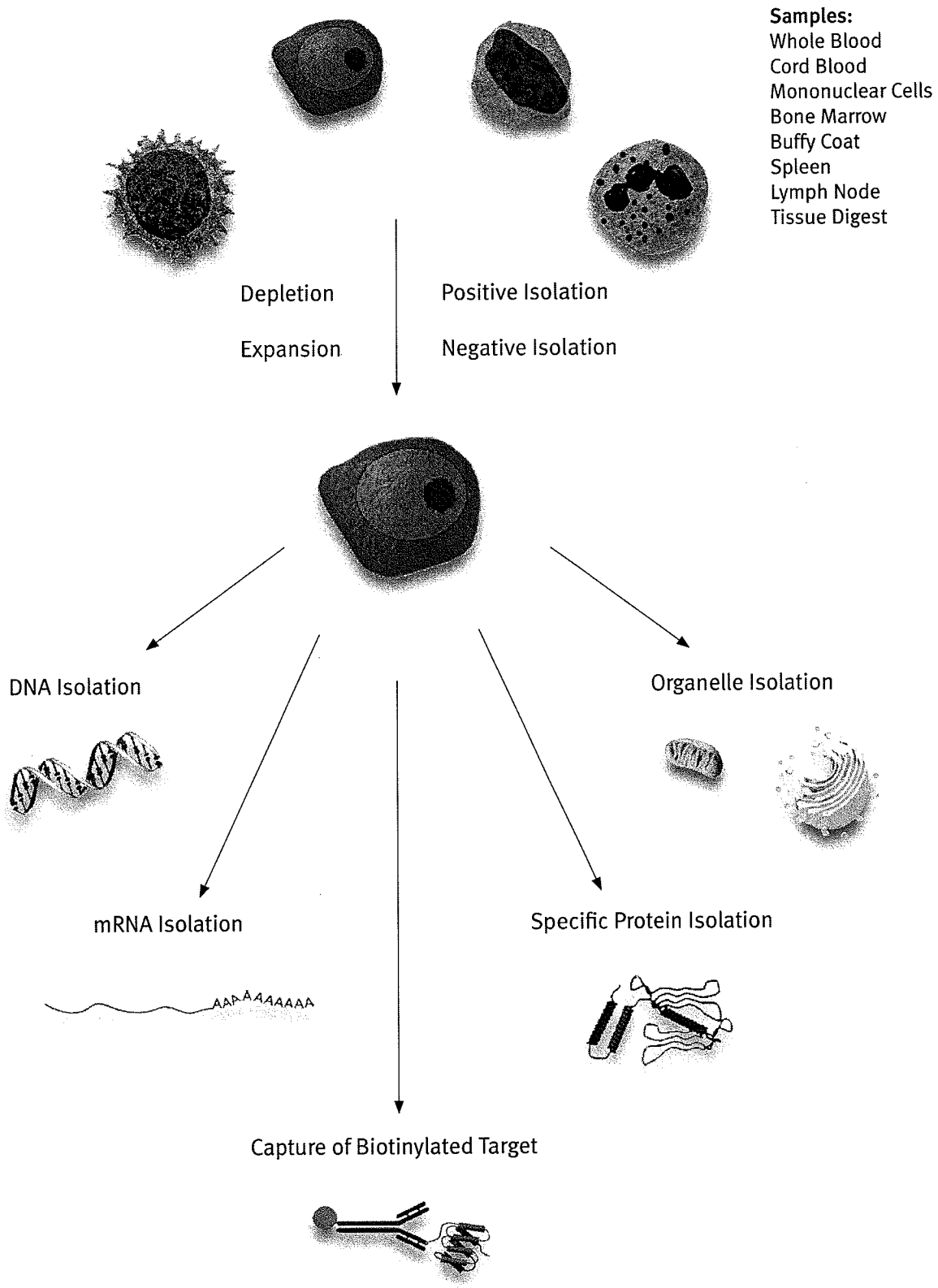
2. The Principles of Dynabeads®

EXHIBIT "B"
Serial No. 10/538,498


Samples:
Whole Blood
Cord Blood
Mononuclear Cells
Bone Marrow
Buffy Coat
Spleen
Lymph Node
Tissue Digest
Depletion
Expansion
Positive Isolation
Negative Isolation
DNA Isolation
Organelle Isolation
mRNA Isolation
AAAAAAAAAA
Specific Protein Isolation
Capture of Biotinylated Target

The Principles of Dynabeads®

What Are Dynabeads® ?

Dynabeads® are superparamagnetic, monosized polymer beads (fig. 1). Each bead has an even dispersion of superparamagnetic material (γFe_2O_3 and Fe_3O_4) coated with a thin polymer shell to encase the magnetic material. This provides a specific and defined surface for the adsorption or coupling of various bioreactive molecules (ligands).

The true uniformity of all Dynabeads® within each batch (typical CV<3%) provides consistent physical and chemical properties. Unique batch-to-batch reproducibility (typically within 5%) secures the reproducibility and quality of your results:

- The beads are superparamagnetic: that is, they exhibit magnetic properties only when placed within a magnetic field and show no residual magnetism when removed from this field.
- The polymer bead shell protects your target from toxic exposure to iron.
- True uniformity (CV<3%) of size, shape and surface area provides optimal accessibility and reaction kinetics, for rapid and efficient binding.
- The true spherical shape and defined surface chemistry minimise chemical agglutination and non-specific binding.
- The specific characteristics of the many available bead types facilitate magnetic separation of a wide variety of targets.
- The long shelf-life of Dynabeads® means you can be sure of great results for many months.

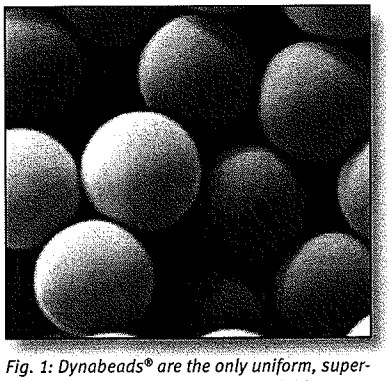


Fig. 1: Dynabeads® are the only uniform, superparamagnetic magnetic beads available.

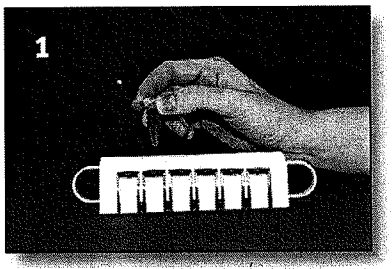


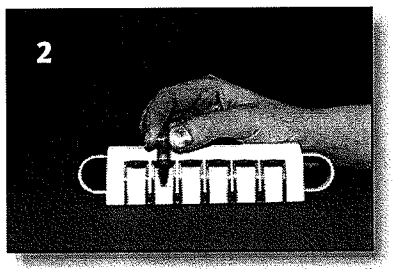
Fig. 2: After target-bead complexes have formed, place the tube in a magnet (Dynal MPC®)

How Are Dynabeads® Used ?

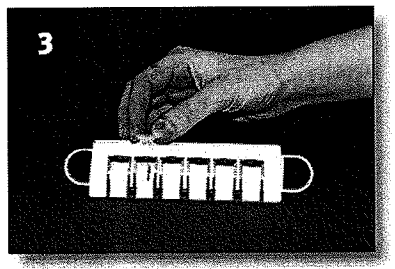
When added to a heterogeneous suspension, Dynabeads® will bind to your desired target (cells, nucleic acids, proteins or other biomolecules). This interaction is based on the specific affinity of the ligand on the surface of Dynabeads®. The resulting target-bead complex can be removed from the suspension using a magnet (Dynal MPC®). Bead-captured complexes are drawn to the side of the tube nearest the magnet, and the supernatant is removed with a pipette. The separation method is gentle, with no need for centrifugation or expensive columns (fig. 2).

Magnetic handling allows you to wash, separate and concentrate the target easily, without centrifugation or columns. The lack of magnetic remanence combined with excellent dispersion abilities also meet the demands of automated systems and microfluidics.

Dynabeads® offer a vast number of possible uses. Dedicated kits have been designed for some applications, whereas other applications can be approached in several ways with different Dynabeads®. Regardless of which Dynabeads® product you choose, you are assured of a superior product with excellent performance and reproducibility.



Within seconds, the target-bead complexes will migrate to the tube-side adjacent to the magnet.



Once the separation is complete, the supernatant can be aspirated and the process repeated to wash the target-bead complexes.

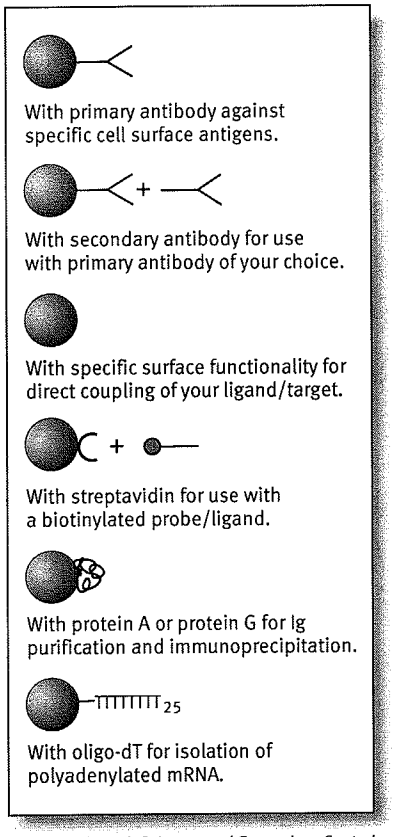


Fig. 3: Activated, Primary and Secondary Coated Dynabeads ® are available.

Different Types of Dynabeads®

Dynal Biotech supplies uncoated Dynabeads® as well as Dynabeads® pre-coated with ligands for specific applications (fig. 3). The ligand can be an antibody, protein or antigen, DNA/RNA probe or any other molecule with an affinity for the target for isolation. The bead-size and the ligand determine the application. For information on tailor-made Dynabeads®, see chapter 7.

Activated, Primary and Secondary-Coated Dynabeads®

Dynabeads® biomagnetic technology utilises affinity interactions between bead-bound ligands and their specific targets. The available Dynabeads® products fall into two categories:

- "Ready-to-use" Dynabeads® are pre-coated with ligands specific for common targets such as cells, proteins or nucleic acids. Dynabeads® for cell separation are conveniently pre-coated with high quality, purified, monoclonal antibodies specific for a wide range of cell surface markers. Dynabeads® are also supplied pre-coated with oligo-dT (for mRNA isolation), with protein A and G (for immunoprecipitation) and with streptavidin (for isolating biotinylated ligands/targets).
- Surface-activated and secondary-coated Dynabeads® are available to allow maximum flexibility of target isolation. Different surface-activated Dynabeads® all have specific physical and chemical functionalities that allow direct coupling of your ligand. Secondary-coated Dynabeads® are pre-coated with purified antibodies, to which you can add a specific antibody of your own choice to isolate your specific target.

Small and Large Dynabeads®

The larger, hydrophobic 4.5 µm Dynabeads® (M-450) are primarily used for cell separation and cell stimulation. The size and magnetic susceptibility of Dynabeads® make them ideal for viscous samples such as whole blood, bone marrow and buffy coat.

The slightly hydrophobic M-500 Dynabeads® also measure 4.5 µm and are used for subcellular fractionations. The ultra-smooth surface of these beads allows for gentle separation of organelles for electron microscopy.

The smaller 2.8 µm Dynabeads® (hydrophobic M-280 and hydrophilic M-270) are used for a wide variety of molecular manipulations, affinity isolations and bioassays, where the beads act as solid-phase during capture, handling and detection.

The new 1 µm Dynabeads® (MyOne™) have an increased surface area per unit weight compared to the larger beads. This high capacity, hydrophilic bead is designed for the *in vitro* diagnostics (IVD), high throughput, routine market. Additionally, these Dynabeads® can be used in a wide range of different molecular applications.

Physical characteristics of Dynabeads®

	Dynabeads® M-450	Dynabeads® M-500	Dynabeads® M-280	Dynabeads® M-270	Dynabeads® MyOne™
Diameter	4.5 µm	4.5 µm	2.8 µm	2.8 µm	1.0 µm
Surface area	1-4 m^2/g	~ 1 m^2/g	4-8 m^2/g	2-5 m^2/g	~ 10 m^2/g
Density	1.6 g/cm^3	1.5 g/cm^3	1.4 g/cm^3	1.6 g/cm^3	1.8 g/cm^3
Iron content	20%	16%	12%	14%	26%

Different Separation Strategies

There are a variety of method options for biomagnetic separation, depending upon the type of target and the specific downstream application. All Dynabeads® methods are gentle to your target and offer the same reproducibility and sensitivity. The different separation strategies are outlined in the following sections.

Positive Isolation: Binding Your Target of Interest

You can positively isolate specific targets from a heterogeneous starting sample by using Dynabeads® coupled with a ligand with an affinity for your target (fig. 4). This flexible system allows you to isolate almost any target, depending on the specific ligand employed. Some typical examples of isolated targets are:

- Specific cell-types and subpopulations of cells
- Subcellular compartments
- Proteins and antigens
- mRNA and genomic DNA
- Specific DNA/RNA fragments

For many applications, it is unnecessary to remove the beads. Instead, the bead-bound target can be used directly in your downstream application.

For downstream applications such as flow cytometry and certain functional studies, Dynabeads® should be detached from the isolated cells. There are two systems available to detach Dynabeads® from positively isolated cells:

- **DETACHaBEAD®** is a patented technology unique to Dynal Biotech. Specific polyclonal anti-Fab antibodies are added to the bead-bound cells and compete with antibody/antigen binding at the cell surface. This releases the antibody and bead from the cells, leaving the target cells viable, unstimulated and without antibody on their surface.

- **CELLection™ Positive Isolation System with Universal Detachment** is a specifically designed system in which the antibodies are attached to the bead-surface via a DNA linker. This linker region provides a cleavable site for removal of the beads from the cells after isolation. Captured cells are gently released by adding the DNase Releasing Buffer supplied with each kit.

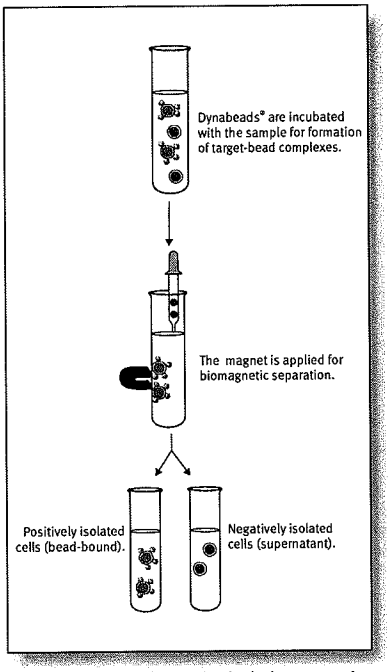


Fig. 4: Positive and negative isolation strategies for separation of specific targets.

Negative Isolation: Untouched Target by Depleting Unwanted Material

Specific targets can also be separated by negative isolation. In this approach, the target of interest is obtained by removing the unwanted component(s) present in a mixed starting sample (fig. 4). The supernatant containing your untouched target is recovered for downstream analyses and assays.

The ready-to-use Dynal Biotech Negative Isolation Kits contain optimised Antibody Mixes plus Depletion Dynabeads® to remove unwanted cells from samples, leaving behind viable and untouched human or mouse cells. You can create your own negative isolation kits for other cell types or specific molecules by adding mixtures of your own antibodies/ligands to secondary-coated or surface-activated Dynabeads®.

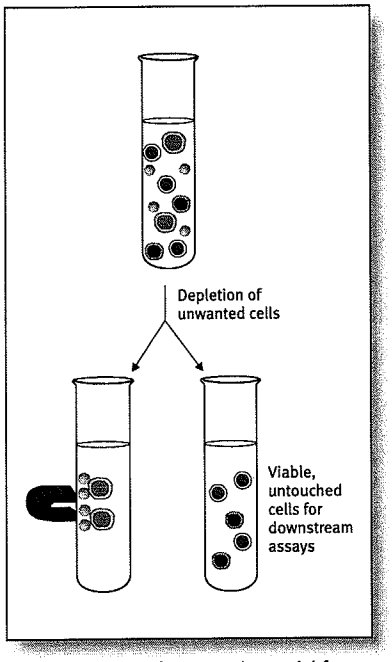


Fig. 5: Depletion of unwanted material from a mixed starting sample.

Depleting Unwanted Material

Specific unwanted material can be easily depleted from a sample (fig. 5). This is done by targeting the unwanted component using Dynabeads® (see page 11). For example, an unwanted cell-type can be removed from a mixed cell sample, or an unwanted protein can be removed from a crude cell lysate. The depleted supernatant - still containing your target - is then recovered for downstream analyses and assays.

Direct / Indirect Capture of Target

The different separation strategies described above can be used with different types of Dynabeads®. Additional separation strategies can be applied with the surface-activated and secondary-coated Dynabeads®:

- In the **direct** approach, the Dynabeads® are simply coated with the target-specific ligand and then added to the sample. This is the most frequently used approach. In cell separation, less primary antibody is consumed when direct capture is used and it can be useful to prepare and store a stock of antibody-coated beads.

- In the **indirect** approach, the ligand is first added to the sample. The resulting ligand-target complex is then incubated with the Dynabeads® for capture. This approach is more effective at cell separation when antigen expression is low. It is possible to custom-make your own negative isolation kit using monoclonal antibodies of your choice. The indirect approach can also be used to minimise unspecific binding when the ligand-target kinetics are slow or the affinity weak. This is of benefit if the ligand concentration is low or the ligand-target binding requires optimal ligand orientation and true liquid-phase kinetics.

Magnetic Handling of Your Target

Biomagnetic separation using Dynabeads® allows for sensitive and reliable capture of specific cells, proteins, genetic material and other biomolecules. Quality of sample preparation is superior, enabling excellence in the results produced during downstream analysis.

Isolating Cells

Antibody-coated Dynabeads® have traditionally been used for isolation or depletion of cells from mixed starting samples. Cell populations separated include T cells, B cells, NK cells, monocytes, dendritic cells, stem cells, stromal cells and cancer cells. Starting samples include whole blood, bone marrow and cord blood or prepared samples such as buffy coat, MNC and tissue digests. With this gentle and efficient separation method it is possible to achieve 99% purity, 99% viability and >95% yield of target cells.

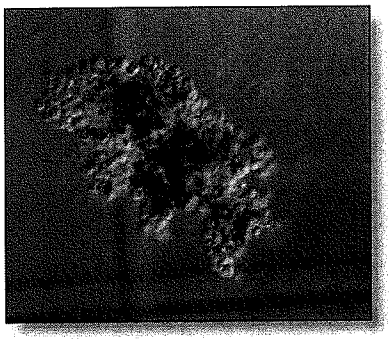


Fig. 6: Human T Cell clones expanded with Dynabeads®.

Immunostimulation of Your Target Cells

Dynabeads® can also be used as artificial antigen-presenting cells to mimic *in vivo* cell signalling for full activation of mouse and human T cells in culture. This cell stimulation is possible with Dynabeads® as their size (4.5 µm) is similar to that of cells. The ready-to-use Dynabeads® present simultaneous signals to TCR/CD3 and CD28 in mouse and human models of human disease (see pages 25 and 43).

Isolating Molecular Material from Specific Cells

Pure cell populations are becoming increasingly used for molecular analyses. Data from homogeneous cell samples are more informative than that from larger samples of mixed cells. Availability of pure cell populations is a challenge in genomic, transcriptomic (fig. 7) and proteomic profiling. Also, due to regulatory mechanisms at the transcriptional, translational and post-translational levels, mRNA and protein levels do not always correlate. Concurrent analysis of both mRNA and proteins from the same sample is required. Throughout this catalogue, Dynabeads®-based technology allows the combined isolation of pure cells, nucleic acids and proteins from single samples (patent pending). This enables downstream molecular characterisation down to single-cell profiling.

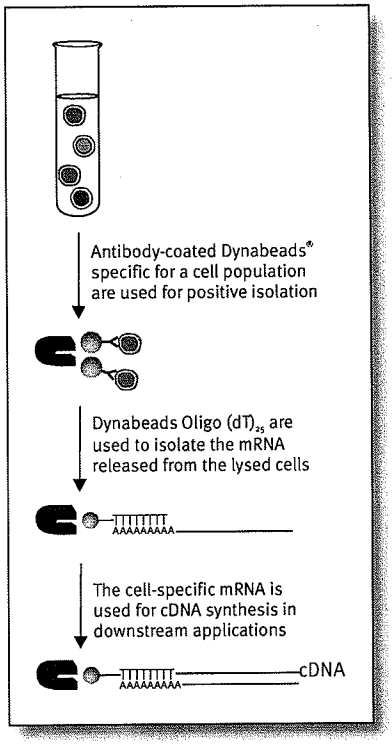


Fig. 7: Positive isolation of a specific cell type, followed by mRNA isolation and further downstream magnetic manipulations.

Downstream Handling of Your Target:

For some applications you need to elute the target from the Dynabeads® prior to downstream analysis. For other applications, the biomagnetic technology also offers a unique benefit in downstream handling of the target. Concentration of target, washes and buffer-changes is easy with a magnet. As an inert solid-phase, the Dynabeads® frequently act as a "carrier" of biomolecules:

- Bead-bound cells are used for further molecular applications, depletion, cell cultures and electron microscopy techniques.
- Bead-bound immunoglobulins are used for target immunoprecipitation and downstream analyses.
- Biotinylated double-stranded DNA is easily converted to single-stranded DNA while bound to streptavidin-coupled Dynabeads®.
- Bead-bound nucleic acids can be further manipulated and subjected to a wide variety of analyses while still attached to the beads.
- Enzymatic reactions are not inhibited by Dynabeads®. This allows for a wide range of molecular assays and applications to be performed directly on the solid-phase (fig. 7).
- Bead-bound material can be included in downstream magnetic handling as well as detection assays.

Automation of Biomagnetic Separation

Not only does magnetic handling eliminate time- and labour-intensive steps, the excellent dispersion abilities and lack of magnetic remanence of Dynabeads® meet the demands of automated systems and microfluidics. Biomagnetic separation has advanced from simple bind-and-separate systems to sophisticated platforms that automate liquid sample preparation, handling and analysis (fig. 8).

Dynabeads® products and protocols are used on a wide variety of liquid handling platforms: e.g. the Isolex® 300i Magnetic Cell Selection System from Baxter Healthcare Corporation, the Biomek® Systems from Beckman Coulter Inc, the Tecan Systems from Tecan AG, the Magnatrix™ Systems from Magnetic BioSolutions AB and the KingFisher® from Thermo Labsystems Oy.

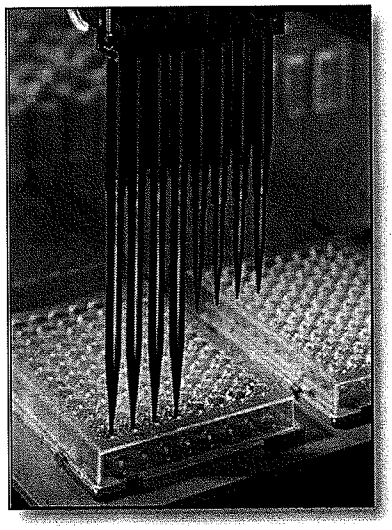


Fig. 8: Different Dynabeads® products and protocols are utilised in a variety of automated laboratory procedures for sample preparation, handling and detection.